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RESEARCH ABSTRACTS OF 1977

Approved and released by:

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Captain, DC, USN
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American Association for Dental Research, General Session, Las Vegas, Nevada, June 23-26, 1977

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*Author presenting paper.
†Poster presentation.

Evaluation of Dental Pulp Irritants with a Rabbit Intradermal Test.

D. M. ANDERSON*, L. G. SIMONSON, K. LANGELAND and D. R. JACKOLA.
Naval Dental Research Institute, Great Lakes, IL and University of Connecticut, Farmington, Connecticut

Previous histologic studies have demonstrated that pulpal inflammation can occur even though the bacteria in carious dentin are far removed from the pulp. To determine the presence of inflammatory agents in caries, a rabbit skin test was used as a screening procedure. Separate samples of both sound and carious human dentin were homogenized in phosphate-buffered saline at pH 7.2 (PBS), centrifuged, and then filtered (0.22μ). The supernatants were dialyzed and lyophilized before preparing various dry-weight concentrations of the filtrates in PBS. After I.V. administration of Nembutal and 1% Evans Blue, 0.2 ml injections of the prepared filtrates and PBS alone were placed intradermally into the shaved dorsa of rabbits. The intensity of bluing and the diameter of wheals produced were measured at varied time intervals. After six hours, no vascular reponse was observed at the PBS sites. Both sound and carious dentin filtrates produced wheals but those from carious dentin were significantly larger and of greater color intensity at equal concentrations. The mean wheal diameters were 11.0 mm and 20.5 mm, respectively. One-way analysis of variance indicated that differences in the three group means were significant at $p < .01$. It was concluded that soluble cell-free filtrates from carious dentin elicit a greater vascular skin response in rabbits than filtrates from sound dentin.

Supported by NMR&DC Project No. MR04120.02 0438.

Inorganic Pyrophosphatase of Streptococcus mutans: Purification and Characterization of Activity. G. CLARK*, and V. FRATTALI, Naval Medical Research Institute, Bethesda, MD, Naval Dental Research Institute, Great Lakes, IL and George Washington U., Washington, D. C.

Inorganic pyrophosphate (PPi) is believed to be an inhibitor of decalcification of dental hard structures. Inorganic pyrophosphatase (PPi'ase) from S. mutans may contribute to initiation of decalcification of dental hard structures by hydrolyzing PPi. S. mutans PPi'ase was isolated and characterized to assist in ascertaining its role in decalcification of dental structures. S. mutans, strain K1R, was grown in Todd-Hewitt broth and harvested at 0.5 O.D., 660nm. Harvested cells were disrupted by ultrasonic treatment and K1R PPi'ase was purified by streptomycin sulfate and heat treatment precipitation, DEAE-Sephadex A-50 anion exchange, Sephadex G-150 gel filtration, and hydroxyapatite absorption chromatography. The PPi'ase enzyme was purified 150-fold with 5% yield to homogeneity as demonstrated by acrylamide gel electrophoresis. Molecular weight appeared to be about 75,000 on the basis of gel filtration and the enzyme consisted of two subunits of about 35,000 on the basis of SDS-polyacrylamide gel electrophoresis. The enzyme required a divalent cation for activity. Mg^{2+} stimulated optimum activity at pH 9.0 with a range from 5.0 to 9.5. Co^{2+} stimulated optimum activity at pH 5.35 with a range from 4.5 to 8.0. The enzyme was inactive in the presence of Ca^{2+} and Mn^{2+}. When appropriate divalent cations such as Mg^{2+} and Co^{2+} are available to the enzyme, it appears that K1R PPi'ase would be active under most pH conditions encountered in dental plaque.

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An Improved Selective Medium for the Isolation of S. mutans.
L. F. DEVINE*, T. P. MOOSHEGIAN and I. L. SHKLAIR, Naval Dental Research Institute, Great Lakes, Illinois

Although many selective media have been developed for the isolation of S. mutans, it is difficult to isolate or obtain a pure culture from a plaque sample that contains $\leq 10^3$ of S. mutans together with 10^8 to 10^{10} microorganisms of other species. A medium was developed for determining the population of S. mutans in such samples. Casman Medium Base was modified by adding the following ingredients per liter: NaCl 15g, sucrose 20g, agar 3g, thallium acetate 0.47g, NaF 0.03g, thioglycollic acid 0.6ml, bromcresol purple 0.015g, and crystal violet 0.075g. After autoclaving, the following antimicrobial agents were added to produce the following concentrations per ml: actidione 60 ug, bacitracin 4 units, Na colistimethate 64 ug, spectinomycin HCl 16 ug and Na azide 3 ug. Laboratory strains of serotypes a, b, c, d, e, f, and g were grown on the new selective medium (SA) and compared to growth on Trypticase Soy Agar (TSA) and Mitis-Salivarius Agar (MS). Serotype a strains did not grow on the SA medium. The growth of 3 of 4 serotype b strains were less than 1% on the SA medium when compared to growth on TSA or MS; the other b strain grew 63% of its colonies on the SA medium. The growth on the SA medium of 14 of 15 serotype c, d, e, f, and g strains ranged from 40-110% when compared to growth on MS or TSA. To test the usefulness of the SA medium in isolating S. mutans 500 approximal plaque samples were collected. Each was placed in 2 ml of a holding medium and 0.1 ml plated on the SA medium. Eighty percent of these samples yielded pure cultures of S. mutans demonstrating the usefulness of this medium in isolating S. mutans from a highly mixed flora.

Enzyme Histochemistry of Osteopetrotic Bone Development in the tl Mutant. E. P. LEONARD and J. W. GALICH*. Naval Dental Research Institute, Great Lakes, IL 60088

The tl (Toothless) mutant rat is characterized by an excessive accumulation of bone which appears closely related to the absence of an osteoclastic cell population. The purpose of this study was to compare the enzyme histochemistry of osteopetrotic tl rat bone with that of normal littermates. Twenty-nine animals from 3 litters derived from separate heterogeneous matings (tl/+) were decapitated at 10, 11 or 12 days of age. The heads and hind limbs were either fresh frozen or fixed in glyoxal prior to sectioning in a cryostat. Serial sections were incubated for acid phosphatase, alkaline phosphatase, β-glucuronidase or succinic dehydrogenase (SDH) activity. Areas of osseous development revealed conspicuous differences with regard to acid phosphatase, β-glucuronidase and SDH activity. Intensely reactive osteoclasts were numerous in sections from both long bones and the bones of the skull in the normal animals. Similar reactive cells were not observed in osteopetrotic bone. Mast cells were highly reactive for acid phosphatase in sections from normal littermates but were unreactive in sections from the osteopetrotic animals. The distribution of alkaline phosphatase activity was indistinguishable between specimens from tl rats and their normal littermates. That osteoclasts were not identifiable either morphologically or histochemically within areas of actively developing tl bone offers further evidence that this mutant possess a bone metabolism in which osteoclastic resorption plays no role.

Supported by NMR&DC Project No. MR04120.20-0408.

Some Characteristics of Dextranase-Degradation Products of Water-Insoluble Streptococcal Glucans. B. L. LAMBERTS*, E. D. PEDERSON, W. ROZMAN, L. G. SIMONSON, Naval Dental Research Institute, Great Lakes, IL and I. C. P. SMITH, National Research Council, Ottawa, Canada

It has been reported that the synthesis of water-insoluble glucan from S. mutans enzymatically in the presence of dextranase yielded an essentially linear α-(1→3)-linked product. This study investigated some characteristics of dextranase-degradation products of water-insoluble glucans from S. mutans strains K-1R and OMZ 176. The glucans were incubated with dextranase at 37 C for 5 days, yielding in each case a non-solubilized fraction (N) and a slowly-dialyzable water-soluble fraction (S). The infrared spectrum of N showed strong absorption at 822 cm^{-1} but little absorption at 793 cm^{-1}, whereas the converse was noted for S. The specific rotations of N, S and undegraded glucan (U) were measured in 1 N KOH and in 9:1 DMSO:H_2O. The initial relationship of specific rotations was N>U>S. However, over periods of up to 27 days at room temperature, the positive rotations of the samples in KOH decreased disproportionately until a reversed pattern (S>U>N) resulted, while rotations for samples in the DMSO:H_2O showed little change. Analyses by ^{13}C NMR showed the content of α-(1→3) linkages for the samples to be in N>U>S order. Although N showed a very high content of α-(1→3) linkages, S had a nearly equal content of α-(1→3) and α-(1→6) linkages. These observations support the concept that N contained α-(1→3) linkages mainly in linear configuration, whereas S more likely represented sites of closely-associated α-(1→3) and α-(1→6) linkages in the undegraded glucans.

Supported in part by NMR&DC Project No. MF51.524.012.0012

Sequential Pathogenesis of Periodontal Destruction in the Rice Rat.
E. P. LEONARD* and L. SWING. Naval Dental Research Institute, Great Lakes, Illinois

The purpose of this study was to determine the sequential pathogenesis of the periodontal syndrome in the rice rat by histochemical and histometric analysis. Sixty rice rats (Oryzomys palustris) were provided at weaning with a plaque inducing diet (Diet 2000). Animals were killed at intervals up to 158 days and the mandibles fixed in either formalin or glyoxal. Glyoxal-fixed mandibles were decalcified in EDTA, cut in the mesio-distal plane and incubated for either: 1) acid phosphatase, 2) alkaline phosphatase, 3) β-glucuronidase, or 4) ATPase activities. Histometric measurements were carried out to determine the average height of the remaining bone, relative degree of inflammatory involvement and osteoclastic activity. Inflammation scores revealed a severe involvement at 5 weeks. The infiltrate was composed predominantly of neutrophils and monocytes and was conspicuously alkaline phosphatase positive. At approximately 8 weeks an acid phosphatase-positive infiltrate was observed subjacent to the crevicular epithelium. These reactive cells were primarily macrophages. With increasing age, the periodontal ligament was more reactive for both acid phosphatase and β-glucuronidase. Among those reactive cells within the periodontal ligament were multinuclear giant cells (apparently perivascular) and selected fibroblasts. Osteoclasts were significantly increased in numbers per unit remaining alveolar bone surface in animals on experiment more than 100 days ($p < 0.01$). Alkaline phosphatase activity appeared constant along alveolar bone surfaces indicating there was no lessening of bone formation.

Supported by NMR&DC Project No. MR04120.20-0408.

The Effect of Sodium Fluoride on the Extracellular Glucan Production of Streptococcus mutans. I. L. SHKLAIR*, R. G. WALTER and B. L. LAMBERTS. Naval Dental Research Institute, Great Lakes, Illinois

The loss of extracellular glucan production by Streptococcus mutans reduces its cariogenicity in animals. Since sodium fluoride is an enzyme inhibitor, its effect on S. mutans extracellular glucan production was investigated. Three strains of S. mutans were tested: high, intermediate and low glucan producers. These organisms were grown in a chemically-defined medium with 5% sucrose for 48 hours with the following concentrations of NaF: 0, 1, 5, 10, 25, 50 and 100 PPM. Glucan production was determined by a phenol sulfuric acid procedure, with the amount of extracellular glucan expressed as mg/ml of glucose. Without NaF the high, intermediate and low glucan-producing S. mutans strains produced means of 0.48, 0.36 and 0.14 mg/ml of glucose respectively. The NaF, at any concentration used, did not significantly inhibit the extracellular polysaccharide production of the strains tested even though there was a uniform decrease in the number of organisms with an increasing concentration of NaF. To further test the effect of NaF on extracellular glucan production a preparation of glucosyltransferase from S. mutans strain K-1R was added to a buffered 10% sucrose solution along with the varied amounts of NaF. This solution was incubated at 37°C for 5 and 24 hours, and the amount of glucan produced determined. The NaF did not inhibit the extracellular glucan production at levels as high as 100 PPM. It was concluded that NaF, at a level as high as 100 PPM, did not inhibit extracellular glucan production of S. mutans.

Supported by NMR&DC Project No. MR0412002-6049.

Biochemical Modification of Dextranase to Improve its Oral Therapeutic Properties. L. G. SIMONSON*, B. L. LAMBERTS and D. R. JACKOLA.
Naval Dental Research Institute, Great Lakes, Illinois

This study has explored means to modify dextranase chemically to increase its affinity for tooth surfaces, thus improving its potential application as an oral therapeutic agent. A commercial dextranase and a dextranase purified by ultrafiltration and hydroxyapatite chromatography from Fusarium moniliforme were used. The purified dextranase was found to have a much greater affinity for a hydroxyapatite column than the commercial dextranase, and was therefore selected for studying methods to further increase its binding properties to human tooth surfaces. Phosvitin was used as a carrier protein. Covalent bonding between dextranase and the carrier was catalyzed by ethyl chloroformate (EtC) or hexamethylene diisocyante (HDC). Various ratios of dextranase, carrier and bridging reagent were studied. More than 34% of the initial activity was retained following EtC treatments while more than 78% of the initial activity was retained following HDC treatments. Molecular changes in HDC-treated preparations were observed by SDS-electrophoresis and routine gel electrophoresis. Gel chromatography (BioGel P-150) of the HDC-treated preparation also revealed two distinct dextranase peaks. One peak was unreacted dextranase (M.W. 39,000) and the other peak (M.W. > 150,000) was the modified dextranase. Therefore, it was possible to complex dextranase to a carrier with a high affinity for hydroxyapatite and still retain activity.

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Glucan Production by Human Isolates and Laboratory Strains of Streptococcus mutans. R. G. WALTER*, I. L. SHKLAIR and B. L. LAMBERTS. Naval Dental Research Institute, Great Lakes, IL

It has been determined that the caries-active (CA) recruit contained more sites infected with S. mutans and greater numbers of this microorganism in each site than his caries-free (CF) counterpart (DMFT=0). Therefore, it was of interest to determine whether or not the S. mutans obtained from these two groups differed in their ability to produce extracellular glucans. In addition some laboratory strains were investigated. All bacterial strains were grown in a chemically-defined medium containing 5% sucrose and 0.5% $NaHCO_3$ for 48 hours at 37°C. Glucan production was determined by a phenol sulfuric acid procedure with the amount of glucan expressed as mg/ml of glucose. Thirteen strains isolated from 8 CF recruits and 12 strains from 6 CA recruits have been analyzed for their ability to produce glucans. The CF strains produced mostly low and intermediate amounts of glucan (.11mg/ml-.40mg/ml) while the CA strains produced larger amounts (.46mg/ml-.67mg/ml). The results indicated that the highest glucan producers and the greatest percentage of high glucan producers occurred in the CA recruit compared to the CF recruit. Of the 8 laboratory strains tested, strain 10449 produced the smallest amounts of glucan (.12mg/ml), strains SD-1, GS-5, B-2, AT-10, Ingbritt and LM-7 produced intermediate amounts (.21mg/ml-.38mg/ml) and strain 001K large amounts of glucan (.60mg/ml). Four strains (10449, GS-5, SD-1 and 001K) were tested for their ability to produce soluble and insoluble glucans. Strains 10449, GS-5 and SD-1 produced about twice as much insoluble as soluble glucan whereas strain 001K produced 1/3 as much insoluble as soluble glucan.

Supported by NMR&DC Project No. MR0412002-6049.

Rupture Strength of Healing Gingival Wounds in Miniature Swine.
M. R. WIRTHLIN*, J. E. YEAGER, B. L. LAMBERTS, E. P. LEONARD, E. B. HANCOCK, and R. W. GAUGLER. Naval Dental Research Institute, Great Lakes, Illinois

The objective was to evaluate quantitatively the healing of standard surgical wounds of the gingiva. Flap curettage procedures were done over the facial radicular surfaces of anterior and premolar teeth of 6 miniature swine that weighed 100kg and were 20 months of age. To make the wound, a blade 3mm wide was inserted into the gingival margin, 0.5 to 1mm lateral, and parallel with the tooth and to the depth of the alveolar crest. Vertical relaxing incisions were made on both sides of the blade. The 3mm wide pedicle flap was reflected, the crevicular pocket was lightly probed and the pocket wall excised at the probed depth. Flaps were returned to place with digital pressure. Healing was evaluated at 0, 2, 7 and 14 days postoperative. On the right side the vertical incisions were freshened and rupture strengths (RS) of the healing flaps tested with tensiometers. The flaps were then excised and extracted in cold neutral salt and acetic acid. The extracts and the insoluble residue were analyzed for hydroxyproline to estimate collagen content. On the left side histometric determinations were made of the extent of epithelial (EP) coverage from the gingival margin to the connective tissue attachment. The results were: Unwounded RS 344g, EP complete; 0 Day RS 10g, EP 38%; 2 Day RS 67g, EP 50%; 7 Day RS 191g, EP 87%; 14 Day RS 312g, EP 99%. The increasing strength of gingival flaps correlated with the regeneration of epithelium. No quantitative evidence of new collagen synthesis during the healing periods was demonstrated by the methods used.

NMRDC M0095-0N004-3010.

A Thirty-Nine Week Functional Evaluation of Four Dental Implant Materials. J. E. YEAGER* and G. W. DILS. Naval Dental Research Institute, Great Lakes, Illinois

The most frequently used types of alloplastic materials for dental implants are surgical grade metals, carbons, ceramics and acrylics. The purpose of this study was to functionally evaluate selected materials from each of these four classes. All first molar and first premolar teeth were extracted from 8 adult Macaca fascicularis (irus) monkeys. Three months were allowed for healing of the extraction wounds. Sixty-four artificial root systems of uniform size and shape were implanted at the prepared sites. Equal numbers of surgical grade Ticonium, vitreous carbon, aluminum oxide and acrylic implants were used. Each alloplastic root was covered with a mucoperiosteal flap. Thirteen weeks postoperatively, the superficial surfaces of the implants were surgically exposed to the oral cavity and crowns with posts were cemented. Occlusal equilibration and splinting were also accomplished. At 26 weeks postoperatively, splinting was discontinued for the purpose of having the implants function independently during the final 13 weeks of the study. Clinical and radiographic criteria used were retention/exfoliation, peri-implant sulcus depth, gingival inflammation, infection, mobility and associated radiolucencies. The vitreous carbon and surgical grade Ticonium implants were superior to the aluminum oxide and acrylic root systems in regard to all evaluation criteria.

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These abstracts are a synopsis of research reported in 1977 at various scientific meetings by the scientific staff of the Naval Dental Research Institute and collaborators from other research activities.

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